

Changes of Cyclic AMP Concentration to Isoproterenol after Abrupt Cessation of Propranolol in Rats

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SUMMARY

Because the mechanism of adverse reactions to abrupt cessation of propranolol in patients with coronary heart disease was an enigma, we studied the effect of cessation of propranolol on beta-adrenergic receptor reactivity to catecholamine stimulation. The cyclic AMP concentrations in plasma and left ventricular muscle after the administration of isoproterenol (5 mg/Kg) were measured in rats before, during, 2 days after, and 4 days after of the administration of propranolol (5 mg/Kg). Two days after withdrawal from propranolol, the cyclic AMP concentrations in plasma and left ventricular muscle were significantly increased ($p < 0.005$, $p < 0.01$). Four days after withdrawal from propranolol, the cyclic AMP concentration in plasma was significantly increased ($p < 0.001$). On the other hand, that of left ventricular muscle showed a tendency to have higher value, although, this was statistically not significant. From these results, this study supports that there is a hypersensitivity to adrenergic stimulation after abrupt cessation of long-term propranolol treatment. The explanation of propranolol withdrawal phenomenon most likely lies in the nature of beta adrenergic receptors that become activated during long-term blockade.

Additional Indexing Words:

Propranolol withdrawal syndrome	Beta adrenergic receptor
Hyperadrenergic state	Cyclic AMP
	Isoproterenol

THERE have been warnings that an abrupt cessation of propranolol treatment in patients with coronary heart disease may be hazardous. Slome¹⁾ reported that an abrupt cessation of propranolol was followed by myocardial infarction. Miller et al²⁾ described serious exacerbation of symptoms of coronary heart disease following the sudden withdrawal of propranolol after crossover to placebo during a double blind trial of propranolol in the treatment of angina pectoris.

Today, with the widespread use of propranolol in patients with ischemic

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heart disease and hypertension and the common practice of abrupt cessation of the drug before surgical procedure, this problem assumes major clinical importance. Several mechanisms have been proposed to explain this phenomenon. An intriguing possibility is that beta adrenergic receptor blockade by propranolol results in increased sympathetic stimulation of the heart soon after the drug is discontinued and such a drug-induced physiological state would increase myocardial oxygen consumption and precipitate ischemic events. It is interesting to know whether there is a rebound hyperadrenergic state or not. But there have been very little data either to support or cast doubt upon the possibility of enhanced sympathetic effects after an abrupt cessation of propranolol or other beta adrenergic blocking agents.

This study was designed to more clearly define whether there was a true hypersensitivity of the beta receptor to adrenergic stimulation following an abrupt cessation of the long-term propranolol treatment. We evaluated responsiveness of plasma and myocardial cyclic AMP to beta adrenergic stimulation (isoproterenol) in rats before, during, 2 days after, and 4 days after the administration of propranolol.

MATERIALS AND METHODS

1. Responses of cyclic AMP in plasma to isoproterenol

Twenty-eight male Wistar rats, median weight of 195 Gm (range 180–220 Gm), were used. They were divided into 4 groups: control group, propranolol group, withdrawal 2 days group, and withdrawal 4 days group (Fig. 1). In control group, saline (0.5 ml) was injected subcutaneously for 10 days. In propranolol group, propranolol (5 mg/Kg) was injected for 10 days. In withdrawal 2 days group, propranolol was initially injected for 10 days and afterwards no medication was given for 2 days. In withdrawal 4 days group, propranolol was initially injected for 10 days and afterwards no medication for 4 days. Before and 1 hour after the subcutaneous transfusion of isoproterenol (5 mg/Kg), 100 μ l of blood was drawn from

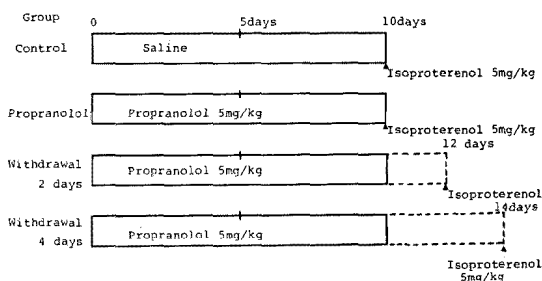


Fig. 1. The experimental protocol of the measurement of cyclic AMP in plasma. In propranolol and withdrawal groups, rats were injected a solution of propranolol, 2 mg/ml in normal saline, at a dose of 5 mg/Kg. In control group, rats were injected similar volume of saline.

tail vein into a heparinized micropipette and immediately diluted with 100 μ l of 10 mM EDTA solution in saline in ice water bath. The solutions were centrifuged (3000 rpm) at 4°C for 15 min and the supernatants were obtained and stocked at -10°C until assay of their cyclic AMP concentrations. The rats in propranolol group were injected isoproterenol 2 hours after the last dose of propranolol.

II. Responses of cyclic AMP in left ventricular muscle to isoproterenol

Twenty-one male Wistar rats weighing about 200 Gm were divided into 3 groups: control group, withdrawal 2 days group, and withdrawal 4 days group (Fig. 2). The rats in control group had no medicine. On the other hand, the rats in withdrawal 2 days group and withdrawal 4 days group were initially given water containing propranolol at approximately 5 mg/Kg of the body weight per day for 10 days and afterwards given water containing no medicine for 2 days and 4 days. The water intake was not influenced by the drug. One hour after the subcutaneous transfusion of isoproterenol (5 mg/Kg) to each group, the rats were decapitated. As soon as the thoracotomy was performed, heart was removed and quickly frozen in liquid nitrogen. A frozen block of heart weighing about 100 mg was homogenized in 2 ml of 6% trichloroacetic acid solution in ice-cold water bath. The homogenates were centrifuged (3000 rpm) at 4°C for 15 min. The supernatant fluid was removed and extracted 3 times with 5 ml of ethyl ether saturated with water. The extracted aqueous phase was stored at -10°C until assay of the cyclic AMP concentrations.

III. Measurement of cyclic AMP

The cyclic AMP concentrations in plasma and left ventricular muscle were measured by a radioimmunoassay of Steiner et al with some modification using an assay kit of Yamasa (Japan).³⁾ To 100 μ l of a sample was added 100 μ l of the dioxane-triethylamine mixture containing succinic anhydride. After 10 min at room temperature, 1 ml of 0.5 M imidazole buffer which contained 0.5% bovine serum albumin, 8 mM theophylline, and 0.01% streptomycin was added to the reaction mixture. To 100 μ l of the mixture were added 100 μ l of [¹²⁵I]succinyl cyclic AMP tyrosine methyl ester and 100 μ l of diluted antisera. The mixture was kept at 4°C for about 20 hours. A cold solution of dextran-coated charcoal (0.5 ml) was added to the mixture cooled in ice-cold water bath. Charcoal was then spun down and 0.5 ml of the supernatant was counted for radioactivity in a gamma spectrometer.

Means and standard errors of the mean were calculated and were compared by the Student's unpaired t-test. The p values of less than 0.05 were considered significant.

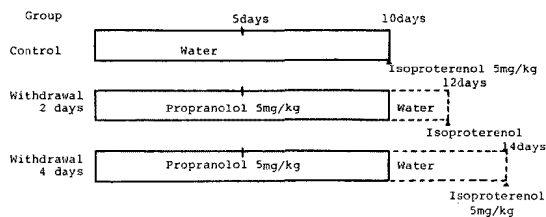


Fig. 2. The experimental protocol of the measurement of cyclic AMP in left ventricular muscle. In withdrawal groups, rats were given water containing propranolol at approximately 5 mg/Kg. In control, rats were given water containing no medicine.

RESULTS

I. Responses of cyclic AMP in plasma to isoproterenol

Fig. 3 shows the means and standard errors of cyclic AMP concentrations in plasma of rats of each group before the administration of isoproterenol. The cyclic AMP concentrations in plasma in control group, propranolol group, withdrawal 2 days group, and withdrawal 4 days group were 74.3 ± 11.5

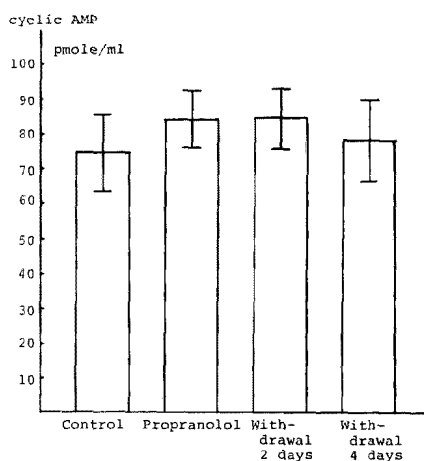


Fig. 3. The cyclic AMP concentrations in plasma before the administration of isoproterenol. Bars represent standard errors of the means. None of the differences between the control group and the experimental groups was statistically significant.

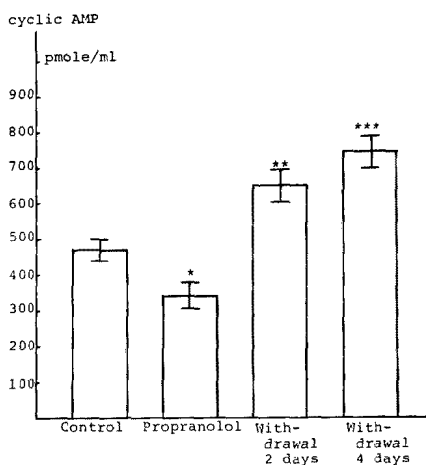


Fig. 4. The cyclic AMP concentrations in plasma after the administration of isoproterenol. Bars represent standard errors of the means. Stars above bars represent statistical significance by unpaired t-test comparing with that value of control (* $p < 0.02$, ** $p < 0.005$, *** $p < 0.001$).

pmole/ml ($n=7$), 84.2 ± 8.5 pmole/ml ($n=7$), 84.6 ± 8.5 pmole/ml ($n=7$), and 78.3 ± 11.5 pmole/ml ($n=7$), respectively. None of the differences among the 4 groups was statistically significant. The plasma cyclic AMP concentrations at 1 hour after the administration of isoproterenol (5 mg/Kg) in control group, propranolol group, withdrawal 2 days group, and withdrawal 4 days group were 472 ± 29 pmole/ml, 342 ± 37 pmole/ml, 648 ± 47 pmole/ml, and 744 ± 45 pmole/ml, respectively (Fig. 4). The cyclic AMP concentrations in plasma after the administration of isoproterenol were significantly increased both in withdrawal 2 days group ($p < 0.005$) and withdrawal 4 days group ($p < 0.001$) as compared with that in control group. But in propranolol group, the plasma cyclic AMP concentrations after the administration of isoproterenol were significantly low as compared with that in control group ($p < 0.02$). The responses to isoproterenol of withdrawal 2 days and 4 days groups were greater than that of control (Fig. 5).

II. Responses of cyclic AMP in left ventricular muscle to isoproterenol

Fig. 6 shows the cyclic AMP concentrations in left ventricular muscle of rats of each group at 1 hour after the injection of isoproterenol (5 mg/Kg). One rat in withdrawal 4 days group died at 20 min after the injection of isoproterenol. The cyclic AMP concentrations in left ventricular muscle after the injection of isoproterenol in control group, withdrawal 2 days group, and withdrawal 4 days group were 1.04 ± 0.03 pmole/mg ($n=7$), 1.35 ± 0.09 pmole/mg ($n=7$), and 1.14 ± 0.08 pmole/mg ($n=6$), respectively. In withdrawal 2 days group, the cyclic AMP concentrations in left ventricular muscle after

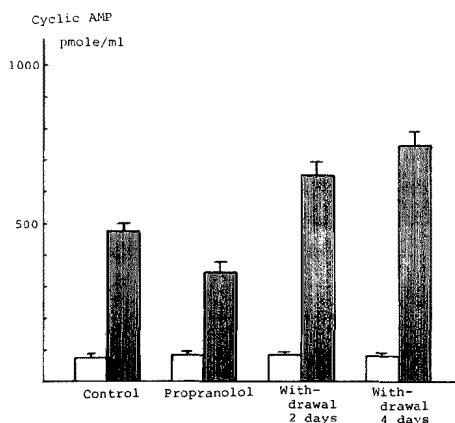


Fig. 5. The responses of cyclic AMP in plasma to isoproterenol. Open left-hand columns represent cyclic AMP concentrations in plasma before the administration of isoproterenol. Closed right-hand columns represent cyclic AMP concentrations in plasma after the administration of isoproterenol. Increases in cyclic AMP after the administration of isoproterenol of 2 days' and 4 days' withdrawal groups are larger than that of control.

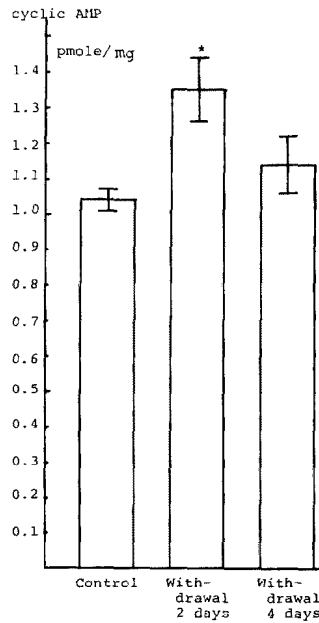


Fig. 6. The cyclic AMP concentrations in left ventricular muscle after the administration of isoproterenol. Bars represent standard errors of the means. Star above bar represents statistical significance by unpaired t-test comparing with that of control (* $p < 0.01$).

the administration of isoproterenol were significantly increased as compared with that in control group ($p < 0.01$). On the other hand, that in withdrawal 4 days group showed a tendency to have higher value. However, this was statistically not significant.

DISCUSSION

The propranolol withdrawal syndrome was first described in a series of case reports.⁴⁾ Miller et al²⁾ noted a 50% incidence of this phenomenon. But most authors now agree that this phenomenon occurs infrequently as compared to previous reports in the literature and Shand and Wood⁵⁾ have described that its incidence is probably 5%. It is also generally agreed that the problems of this phenomenon occurred more often in patients who maintained normal activity than in patients in the hospital.⁶⁾ Several mechanisms have been proposed to explain this phenomenon. One of the suggestions was that the ischemic heart disease process progressed, or that the patients continued to attempt the more strenuous activity that propranolol treatment had allowed. Diaz et al⁴⁾ suggest that beta blockade permits the asymptomatic progression of coronary atherosclerosis while protecting the patient from the

effects of this progression, namely, angina pectoris and possible myocardial infarction, by lowering myocardial oxygen requirements. When beta blockade (propranolol) is abruptly removed and oxygen requirements suddenly increase, the oxygen delivery system is no longer capable of meeting this renewed increase in oxygen demand, resulting in severe angina pectoris or myocardial infarction. Schrumpf et al⁷⁾ reported that propranolol therapy decreased affinity of hemoglobin for oxygen and improved tissue availability of oxygen. Abrupt cessation of propranolol may possibly result in adverse alterations in oxygen affinity for hemoglobin. Another possible explanation of this phenomenon may be related to the effects of propranolol on platelet aggregation.⁸⁾ An alternative explanation has been provided by Alderman et al⁹⁾ who suggest true rebound phenomenon. Up to now, no satisfactory explanation of this phenomenon has been demonstrated and its mechanism is undefined.

Boudoulas et al¹⁰⁾ showed a significant rebound increase in heart rate, pulse pressure, and shortening of electromechanical systole produced by isoproterenol in normal volunteers after 2 days' propranolol administration. Although this theory is an attractive one, negative reports do exist. Myers and Horwitz¹¹⁾ have failed to show increased sensitivity to epinephrine or isoproterenol in dogs. The area of greatest interest has been whether there is a true rebound hyperadrenergic state or not. So, we carried out this study to ascertain from the point of biochemical and pharmacological responses whether existed a hypersensitivity of the beta receptor to adrenergic stimuli after an abrupt cessation of propranolol. Barnes et al¹²⁾ reported that increasing dose of isoproterenol, a potent beta adrenergic receptor agonist, resulted in linear increases in heart rate and dP/dt max, an index of myocardial contractile force. Rall¹³⁾ found a rise in the plasma cyclic AMP concentration after intravenous application of catecholamine. Cyclic AMP is believed to be an intracellular second messenger of catecholamine action. The role of cyclic AMP as a second messenger reflects its ability to translate the initial message generated by the arrival at the cell surface of a hormone (catecholamine) into a second message that can be recognized within the cell. This second message is carried by cyclic AMP, which is produced from ATP by an enzyme called adenylate cyclase. This enzyme is activated when the catecholamine hormones interact with beta receptors located on the cell membrane. Thus, there are some correlations between the inotropic effect of catecholamine and cyclic AMP formation. If there is a true rebound hyperadrenergic state after an abrupt cessation of propranolol, it may be expected that withdrawal of propranolol produces a marked rebound increase of cyclic AMP formation, a reflection of the increased adenylate cyclase and/or there

is a hypersensitivity of the beta receptor to adrenergic stimuli as compared with normal condition. Therefore, the measurements of cyclic AMP concentrations in plasma and left ventricular muscle after the administration of isoproterenol would be expected to provide a reliable means of biochemical assessing cardiac sensitivity to catecholamine. In view of the fact that the most obvious changes shown by Boudoulas et al¹⁰⁾ in man occurred at 36 and 48 hours after an abrupt cessation of propranolol, we measured the cyclic AMP concentrations before and after the administration of isoproterenol at 2 days and 4 days post-withdrawal of propranolol.

In this study, the cyclic AMP concentrations in plasma before the administration of isoproterenol did not show any significant differences among 4 groups. While, the cyclic AMP concentrations in plasma after the administration of isoproterenol increased in all groups, but increases at 2 days and 4 days after withdrawal from propranolol were significantly large. This increased response to isoproterenol could be observed also in left ventricular muscle 2 days after withdrawal from propranolol. On the other hand, that of withdrawal 4 days group showed a tendency to have higher value but this was statistically not significant. According to this study, there is an evidence that an abrupt cessation of propranolol is associated with the development of greater than normal sensitivity to isoproterenol. We have shown that an abrupt cessation of propranolol after chronic administration is followed by transient hypersensitivity to catecholamine. The time course of this phenomenon was short and the response to isoproterenol at 4 days after withdrawal from propranolol returned to control condition.

Our findings of beta adrenergic hypersensitivity after propranolol withdrawal are supported by Boudoulas et al.¹⁰⁾ However, Myers and Horwitz¹¹⁾ have failed to show increased sensitivity to isoproterenol. The most obvious changes shown by Boudoulas and our study occurred around 48 hours after an abrupt cessation of propranolol. Myers performed no measurements between 24 and 72 hours after cessation. Although species variation cannot be excluded, this discrepancy is probably due to time variation of blood sampling.

Possible mechanisms of this hyperadrenergic state include increased number of beta adrenergic receptor binding sites and altered affinity of beta receptors for catecholamines. Glaubiger and Lefkowitz¹⁴⁾ have reported that treatment of rats with propranolol for 2 weeks leads to a 100% increase in the number of beta adrenergic receptors. Our study do not allow a conclusive explanation for mechanism of hyperadrenergic state.

In conclusion, we found that there was a rebound hyperadrenergic state after an abrupt cessation of long-term propranolol treatment. Because our

study was conducted in rats, our results may not be directly translated to patients with coronary heart disease. But, this finding may provide a possible explanation of the clinically observed propranolol withdrawal syndrome.

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